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METHYLAURONES FROM *CYPERUS CAPITATUS*

ROSA M. SEABRA*†, ARTUR M. S. SILVA‡, PAULA B. ANDRADE† and M. MANUELA MOREIRA†

† CEQUP/Lab Farmacognosia, Fac Farmácia da U.P., R. Aníbal Cunha, 4050 Porto, Portugal;
‡ Chemistry Department, University of Aveiro, 3810 Aveiro, Portugal

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Key Word Index—*Cyperus capitatus*; Cyperaceae; flavonoids; C-methylaurones; structure revision; 4,6,3′,4′-tetrahydroxy-5-methylaurone; 4,6,3′,4′-tetrahydroxy-7-methylaurone; 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone; 6,3′,4′-trihydroxy-4-methoxy-7-methylaurone.

Abstract—4,6,3′,4′-tetrahydroxy-5-methylaurone, 4,6,3′,4′-tetrahydroxy-7-methylaurone and 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone were isolated from *Cyperus capitatus*. On the basis of NMR studies, the structures of the methylaurones previously isolated from this species were revised and the compound described before as 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone is shown to be 6,3′,4′-trihydroxy-4-methoxy-7-methylaurone.

INTRODUCTION

Only three methylaurones have been described so far. The first compound of this class is 6,4′-dihydroxy-7-methylaurone-6-*O*-rhamnoside, isolated from *Pterocarpus marsupiam* [1]. The other two methylaurones described are non-glucosidic and were isolated from *Cyperus capitatus* Vandelli [2, 3]. On the basis of MS, ^{1}H and ^{13}C-data they were identified as 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone (**a**) [2] and 6,3′,-dihydroxy-4,4′-dimethoxy-5-methylaurone (**b**) [3]. Continuing our study of this same species, we isolated three further methylaurones, which make five compounds of this kind in *C. capitatus*. Two of the compounds (**1** and **2**) were aureusidin based, having a methyl group on C-5 and on C-7, respectively. The third compound (**3**) showed ^{1}H and ^{13}C NMR spectra not significantly different from those described for **a** previously isolated from *C. capitatus*, which leads us to think that the structure first ascribed might be incorrect with regard to the position of the methyl group. Further studies were therefore necessary in order to guarantee the correct structural assignment of the compounds.

The same doubt was valid for the structure later assigned to **b**, since the reasoning for its structural assignment was based on the data obtained for compound **a**. Structure corrections are now made, based on unequivocal assignments of all proton and carbon resonances, using 2D COSY and HETCOR experiments and also a one-dimension selective INEPT measurements [4].

RESULTS AND DISCUSSION

Two further compounds were isolated, **1** and **2**, from the methanolic extract of *C. capitatus*. These compounds had the same R_f value in 60% acetic acid (TLC, cellulose), but **1** had a higher R_f value in BAW, suggesting less polarity than **2**. They were both fluorescent yellow under UV light (366 nm) and turned orange on exposure to ammonia vapour or when sprayed with N A (Naturstoffreagenz A) on TLC cellulose plates. Chromatographic behaviour (R_f, R_f, colours) remained unchanged after heating with HCl. They had UV–vis spectra typical of aurones having *ortho*-dihydroxyls on the B-ring and a hydroxyl on C-4. Both had the same mass spectra. Their ^{1}H NMR spectra were very similar, exhibiting the pattern of aurones of the aureusidin type [5] bearing a methyl group on A-ring: three signals (1H each) with the shifts and splitting of a catechol B-ring, two singlets (1H each) for a benzylic proton and a A-ring proton and a singlet (3H) for a methyl group. On the basis of these data, **1** and **2** were shown to be 5-*C*-methylaureusidin and 7-*C*-methylaureusidin.

The third compound (**3**) was fluorescent yellow under UV light (366 nm) and turned orange on exposure to ammonia vapour or when sprayed with N A on TLC plates. Chromatographic behaviour (R_f, R_f, colours) remained unchanged after heating with HCl.

* Author to whom correspondence should be addressed.

It had a UV–vis spectrum typical of an aurone having a catechol type B-ring and lacking an hydroxyl group *peri* to the carbonyl group since the complex formed with $AlCl_3$ was acid labile [6]. ^1H and ^{13}C NMR spectra confirmed the catechol type B-ring and, as in **1** and **2**, it showed the most common C-4–C-6 oxidation pattern; a methoxyl signal is observed which must be located on C-4 given the lack of a stable complex with $AlCl_3$; a methyl group (δ 1.96, *s*, 3H) and a signal for one proton (δ 6.41, *s*, 1H) are also observed which must be located on the A-ring. These data are similar to those previously reported for the first methyl aurone isolated from this species (compound **a**). Since **a** and **3** have different R_f and R_t values, but the same mass spectra, they must be isomers, one of them having a methyl group on C-5 and the other with a methyl group on C-7. When compound **a** was isolated, the methyl group was assigned to C-5 position on the basis that a methyl group in this position would provoke a sterical hindrance in the neighbouring methoxyl group which would show a δ value around 60, while if no methyl group is linked to C-5, the methoxyl group would have a δ of about 55–56 [7]. Unexpectingly the two *C*-methylaurones in question have similar ^1H and ^{13}C NMR spectra in contrast to the two isomeric corresponding methylflavanones isolated from *Pityrogramma pallida* [8].

In order to determine exactly where the methyl groups were located in each compound we have made some one-dimensional selective INEPT measurements. In the case of **3**, on irradiation of the methoxyl proton resonance, optimised for 7 Hz long-range *J* (C,H) coupling, enhancement on the resonance at δ 156.8 ppm was observed. Subsequent to irradiation of the methyl proton resonance, in the same conditions, enhancements were observed on the signals at δ 111.6, 156.8 and 166.3 ppm, which were attributed to the C-5, C-4 and C-6 carbon resonances, respectively. Thus **3** is identified as 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone while the structure of **a** must be corrected to 6,3′,4′-trihydroxy-4-methoxy-7-methylaurone (**4**).

When **b** was isolated, the structure of its A-ring was thought to be 4-methoxy-5-methyl-6-hydroxy on the basis that the ^1H and ^{13}C NMR data were similar to that of A-ring of **a**. Once we verified that ^1H and ^{13}C NMR data were not enough for a safe assignment of *C*-methyl groups, the structure of **b** was also revised. In this case we have also carried out some one-dimensional selective INEPT measurements [4] with long-range *J* (C,H) coupling constants optimised for 7 Hz. On irradiating both of the methoxyl (δ 4.06 and 3.83 ppm) protons resonances, enhancements were observed on the signals at, respectively, δ 157.0 and 149.5 ppm. Upon irradiation of the methyl proton resonances, enhancements were observed on the signals at δ 111.7, 157.0 and 165.4, which were assigned to C-5, C-4 and C-6, respectively. These data prove that we have a 4-OCH_3–5-CH_3–6-OH substitution pattern in the A-ring. That of B-ring was established

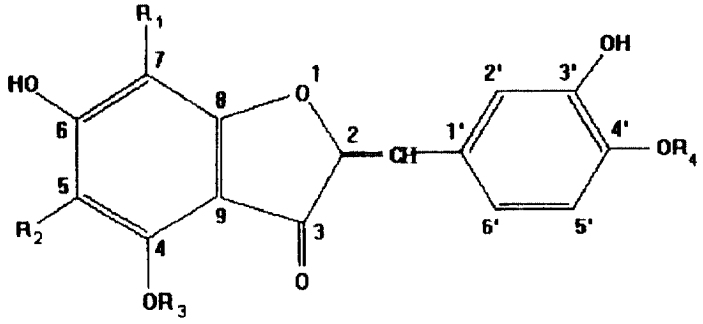
Table 1. ^1H NMR and chromatographic data of compounds 1–5 (DMSO-d_6, TMS as int std)

Position	1	2	3	4	5
5		6.15 (*s*, 1H)	—	6.54 (*s*, 1H)	—
7	6.20 (*s*, 1H)	—	6.41 (*s*, 1H)	—	6.69 (*s*, 1H)
benzylic	6.40 (*s*, 1H)	6.41 (*s*, 1H)	6.47 (*s*, 1H)	6.54 (*s*, 1H)	6.60 (*s*, 1H)
2′	7.40 (*d*, 1H, J = 1.7 Hz)	7.44 (*d*, 1H, J = 1.6 Hz)	7.40 (*d*, 1H, J = 1.9 Hz)	7.41 (*d*, 1H, J = 1.9 Hz)	7.48 (*d*, 1H, J = 1.8 Hz)
5′	6.80 (*d*, 1H, J = 8.2 Hz)	6.81 (*d*, 1H, J = 8.2 Hz)	6.81 (*d*, 1H, J = 8.2 Hz)	6.82 (*d*, 1H, J = 8.3 Hz)	7.02 (*d*, 1H, J = 8.2 Hz)
6′	7.14 (*dd*, 1H, J = 1.7; 8.1 Hz)	7.16 (*dd*, 1H, J = 1.5; 8.1 Hz)	7.17 (*dd*, 1H, J = 1.8; 8.2 Hz)	7.19 (*dd*, 1H, J = 1.8; 8.2 Hz)	7.31 (*dd*, 1H, J = 1.8; 8.2 Hz)
	1.89 (*s*, 3H, 6-CH_3)	2.10 (*s*, 3H, 7-CH_3)	1.96 (*s*, 3H, 5-CH_3)	1.97 (*s*, 3H, 7-CH_3)	1.89 (*s*, 3H, 5-CH_3)
			4.03 (*s*, 3H, 4-OCH_3)	4.04 (*s*, 3H, 4-OCH_3)	4.06 (*s*, 3H, 4-OCH_3); 3.83 (*s*, 3H, 4′-OCH_3)
R_f (BAW)	0.60	0.47	0.85	0.63	0.81
R_f (AcOH 60%)	0.21	0.21	0.52	0.41	0.60
R_t	19′ 20″	15′ 10″	23′ 40″	15′ 10″	28′ 50″

Table 2. ^{13}C NMR data of compounds 3–5 (DMSO-d_6, TMS as int std)

Position	3	4	5
2	145.6*	145.5*	146.0
3	178.5	178.8	178.8
4	156.8	156.9	157.0
5	111.6	93.3	111.7
6	166.3	165.1†	165.4
7	93.4	111.4	93.4
8	165.7	165.6†	165.8
9	105.0	105.5	105.5
benzylic	110.6	110.9	110.5
1′	123.6	123.6	124.9
2′	117.6	117.7	117.2
3′	145.7*	145.6*	146.7
4′	147.8	147.8	149.5
5′	116.1	116.1	112.3
6′	124.2	124.3	124.1
	8.3 (5-CH_3)	8.3 (7-CH_3)	8.3 (5-CH_3)
	61.3 (4-OCH_3)	61.4 (4-OCH_3)	61.6 (4-OCH_3)
			55.7 (4′-OCH_3)

†,* Assignments with the same superscripts may be interchanged.



1 R_2=Me, R_1=R_2=R_4=H

2 R_1=Me, R_2=R_3=R_4=H

3 R_2=R_3=Me, R_1=R_4=H

4 R_1=R_3=Me, R_2=R_4=H

5 R_2=R_3=R_4=Me, R_1=H

by irradiation of the OH proton resonance (δ 9.35 ppm) which gave enhancements on the signals of C-2′ (δ 117.2 ppm) and those at δ 149.5 and 146.7 ppm. These results allow us to assign unequivocally the C-3′ (δ 146.7 ppm) and C-4′ (δ 149.5 ppm) resonances and the corresponding B-ring substituents (3′-OH,4′-OCH_3). Hence **b** is correctly identified as 6,3′-dihydroxy-4,4′-dimethoxy-5-methylaurone (**5**).

In the first two publications, the attribution of δ values of B-ring was based on values published for sulfuretin and flavonoids with 4′-methoxy groups [7]. On revising these structures the 2D NMR COSY and HETCOR experiments of **3** and **5** were also recorded. From the spectra, the δ values of B-ring and benzylic carbons can now be correctly attributed.

EXPERIMENTAL

Plant material, extraction and general methods

Isolation and purification as in [2]. Isolation was achieved by column chromatography with Sephadex LH-20 and RP C18 Lobar, eluting with MeOH and MeOH 90%.

Analytical HPLC. Methanolic extracts were analysed using a RP Spherisorb ODS2 column (25

cm × 4.5 mm; 5μm) using water:formic acid (solvent A) and methanol (solvent B), at room temperature with a flow rate of 1.0 ml min^{-1}, with the following gradient: 0′–40%B, 5′–45%B, 10′–55%B, 20′–60%B, 32′–100%B, 36′–100%B. Detection was made by a diode array. In these condition R_f were those indicated in Table 1.

Compound 1. UV–vis λ^{MeOH} nm: 256, 272, 395; + NaOMe: 290, 379 *sh*, 460 (slow decreasing intensity after 5 min); + $AlCl_3$: 262, 285, 343 *sh*, 370 *sh*, 509; + $AlCl_3$ + HCl: 256, 279 *sh*, 408, 455. EIMS *m/z* (rel. int.) $C_{16}H_{12}O_6$ (Found: 300.0615; Calc.: 300.0630) $[M]^+$ (100), 299 $[M-1]^+$ (48), 271 $[M-HCO]^+$ (14), 166 $[A_1]^+$ (24), 167 $[A_1+H]^+$ (58).

Compound 2. UV–vis λ^{MeOH} nm: 259, 269, 346, 409; + NaOMe: 283, 369 *sh*, 452 (slow decreasing intensity after 5 min); + $AlCl_3$: 252, 266 *sh*, 348, 521; + $AlCl_3$ + HCl: 267, 323 *sh*, 400, 468. EIMS *m/z* (rel. int.) $C_{16}H_{12}O_6$ (Found: 300.0612; Calc.: 300.0630) $[M]^+$ (91), 299 $[M-1]^+$ (43), 271 $[M-HCO]^+$ (12), 166 $[A_1]^+$ (34), 167 $[A_1+H]^+$ (100), 134 $[B_1]^+$ (21).

Compound 3. UV–vis λ^{MeOH} nm: 257, 273 *sh*, 339 *sh*, 400; + NaOMe: 275, 445 (slow decreasing intensity after 5 min); + $AlCl_3$: 261, 286 *sh*, 338 *sh*, 434; + $AlCl_3$ + HCl: superimposable to that of MeOH. EIMS *m/z* (rel. int.) $C_{17}H_{14}O_6$ (Found: 314.0773; Calc.: 314.0786) $[M]^+$ (95), 314 $[M-1]^+$ (45), 285 $[M-HCO]^+$ (15), 180 $[A_1]^-$ (34), 181 $[A_1+H]^+$ (100), 134 $[B_1]^+$ (30) (notation according to Ref. [9]).

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